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Repair of Body Wall Incision in the Rhynchobdellid Leech *Piscicola salmositica*[1-3]

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Leeches were wounded in the posterodorsal area by nonsterile incision. Initial response consists of bodily contraction and localized constriction, resulting in the forcing of injured tissue and debris toward the lesion and the expelling of some of the debris to the exterior. Undifferentiated cells immigrate into the area and form a connective tissue plug incorporating the remaining debris. The debris is expelled by a continuous sloughing of the outer plug cells, while recruitment of additional immigrating cells replenishes the plug. A weak phagocytic response is noted if a major coelomic channel is severed. After the wound is cleared of debris, the connective tissue mass is covered by migration of adjacent epithelial cells. No additional repair occurs for at least the following 6 days, and only a scar remains.

INTRODUCTION

The wound repair process in the class Hirudinea of the Annelida has received little attention, possibly because it has been overshadowed by the rather phenomenal segmental regenerative ability of the related Polychaeta and Oligochaeta. Myers (1935), however, did describe repair of tissues injured during insemination of the glossiphonid leech *Placobdella parasitica*. In this species the acting male cements a spermatophore to the body of the acting female, and an apparently chemically induced histolysis of the body wall cells permits intrusion of the spermatophore contents. The sperm-laden seminal fluid subsequently makes its way to the "uterus," where fertilization is accomplished, but it leaves a path of cellular destruction and disorganization behind.

Immediately after passage of the fluid, the pathway begins filling with a "plug" essentially composed of three types of cells, which were distinguished by Myers only on the basis of nuclear dimensions. Cells with small oval nuclei, 2.5–4.0 μ long, appear to migrate from adjacent parenchymatous tissue, while larger predominant cells with 5.0–7.0 μ long, "somewhat oval" nuclei are most likely wandering leukocytes which migrate to the area. Some larger phagocytes with spheroid nuclei longer than 7.0 μ are also present. Growth and differentiation of these cells account for regeneration of the tissues, and no mitotic figures could be identified with certainty. Myers noted that tissues injured by other means are not regenerated, but that healing of the seminal channel is probably the specific evolutionary response to this animal's mode of reproduction.

MATERIALS AND METHODS

Leeches were maintained in glass jars containing 300–350 ml of water from a lake that normally supports a population of *Piscicola salmositica*. The water was previously vacuum filtered through Whatman No. 2 paper, and approximately three-fourths of the wa-

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ter in each jar was changed daily by use of a siphon to minimize disturbance of the leech. Because *P. salmositica* is apparently negatively phototaxic (Becker, 1964), the outside of the bottom one-half of each jar was painted black. The jars were kept in a water bath maintained at 10±1°C, and the water in each jar was aerated with an individual air stone, controlled by a separate valve.

Specimens were anesthetized by addition of 4 ml of saturated aqueous magnesium chloride solution to 35 ml of the water containing the leech, in a 42±1°C water bath. They were then fixed for 24 hr at room temperature in a buffered, aqueous 10.0% formalin–1.0% glutaraldehyde solution, and transferred to 50% aqueous isopropyl alcohol. Within 3 days of termination of a given experiment all specimens were processed together using standard histological technique, with Mayer's hematoxylin and eosin staining and Mallory's aniline blue collagen staining used for routine examination of the 7–8μ sections.

Each leech was wounded in the dorsal area of the posterior end, 1–3 segments anterior to the posterior disc. Preliminary histological examination indicated that this area was the least complicated and that specific repair processes would be more clearly discernible here than in more complex areas. A diagonal incision, at an approximate 45° angle from the longitudinal axis, was inflicted with an unsterilized razor blade.

Results

Normal Histology

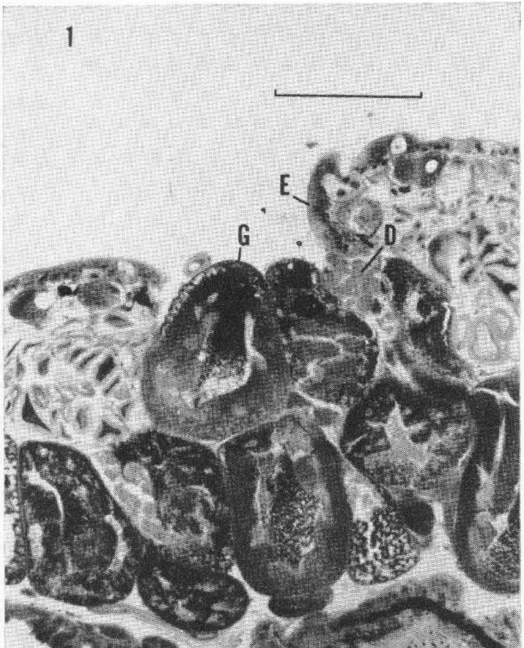
It may be helpful to review the normal histology of tissues corresponding to those wounded in experimental leeches. The outer layer of the body wall consists of a cuticle-covered epithelium of cuboidal to low columnar cells, with centrally to basally located nuclei. Between many epithelial cells are minute capillaries to the exterior, which are reported (Borradaile et al., 1963) to be part of the blood circulatory system, but which appear, in *P. salmositica*, to be contiguous with the coelomic channel network. Beneath the epithelium is a very thin, sometimes locally absent, layer of parenchymatous connective tissue containing many fat or pigment cells, which, in *Glossiphonia complanata*, change from fat cells to pigment cells in the course of development (Bradbury, 1957). In *P. salmositica* the predominant form is the fat cell.

The underlying muscle consists of two layers of smooth muscle, one circular and one longitudinal. The circular muscles form a rather continuous band 2 to 3 cells deep over the entire length of the body, whereas the longitudinal muscles are typically arranged in bundles of 15 to 30 cells each. In cross section, all muscle cells are seen to possess an eosinophilic cortex surrounding an amorphous, basophilic protoplasmic medulla.

Beneath the muscle layers, loose connective tissue contains additional fat cells, cocoon gland cells and associated ducts, coelomic channels and an occasional cross-body muscle fiber. Under the dorsal body wall is the intestine, consisting of a lumen surrounded by a single layer of cuboidal epithelium with prominent nuclei and often with numerous goblet cells. Centrally located and usually most prominent is the stomach, with its ciliated, low cuboidal epithelium and usually containing fish blood and blood corpuscles.

Observations

Because of the rapidity required to inflict incisions, wounds varied considerably in severity. In some cases only the cuticle, epithelium, dermal connective tissue, fat cells, and circular muscles were severed. In others, the incision continued through the longitudinal muscle layer, and in still others the stomach or intestine was cut. The time sequence of wound healing as described here, therefore, lacks significance, but the mechanistic sequence is of value.



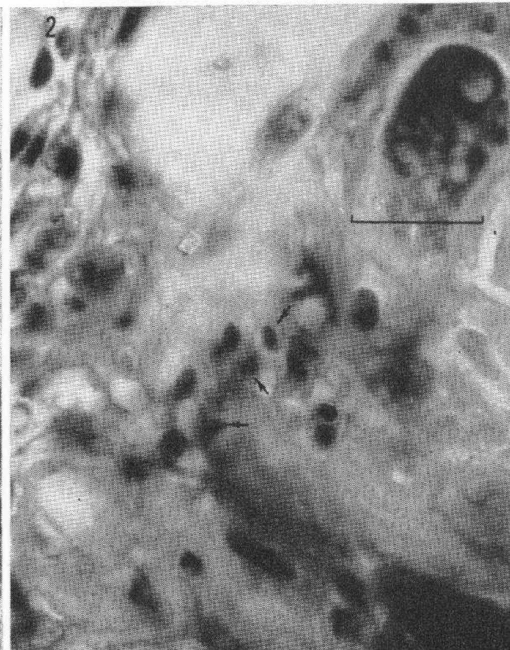


FIG. 1. 1 hr post-injury. Note the cocoon gland cells (G) and associated collecting ducts (D) plugging the wound by closely abutting adjacent uninjured epithelium (E). Line equals 0.150 mm (150 μ).

FIG. 2. Immigrating cells are indicated by arrows. The cell membranes are typically indistinct and often are not visible. Line equals 0.015 mm (15 μ).

Immediately upon wounding, *P. salmositica* contracts and constricts the segments involved. Although the severed ends of the epithelium do not completely close the wound, they so closely approximate each other that tissues squeezed toward the wound by contraction plug the lesion (Fig. 1). Although only a temporary measure, it effectively prevents loss of body fluids.

The first immigrating cells (Fig. 2) appear as early as 1 hr post-injury in the intermuscular spaces and near dermal connective tissue adjacent to the wound. These cells are typically round to slightly oval, with a diameter of 5–6 μ, and have round, centrally located nuclei measuring approximately 2 μ in diameter. The cytoplasm is achromatic to weakly basophilic with hematoxylin and eosin, while the nuclei are densely basophilic. With Mallory's trichrome, however, the cells stain blue and are usually indistinguishable from surrounding tissue. Because cells of this type are never seen anywhere other than in the immediate area of the wound, their early appearance presents a valuable indication of their origin. They appear to arise from uninjured intermuscular and dermal connective tissue.

By 4 hr post-injury a thin bridge of immigrating cells forms across the surface of a shallow wound. A deeper wound, on the other hand, may not be covered for some time, but by 8 hr post-injury the process is well underway, with a sheet typically 9–10 cells in thickness developing inwardly from the wound edges.

At approximately 8 hr post-injury the first phagocytic cells appear, but only when a major coelomic channel is severed. These coelomic phagocytes are typically round, measuring 6–10 μ in diameter, and with hematoxylin and eosin staining exhibit achromatic cytoplasm sometimes containing basophilic inclusions. The deeply basophilic nucleus is located either centrally or eccentrically, and measures 2–3 μ in diameter. Apparently identical phagocytes are normally found in nephridial capsules and are presumed to be capable of carrying phagocytosed materials from the coelomic channels to the capsule via the nephrostome. These cells may also occur in the ventral coelomic channel. No phagocytes were noted in wounds after 16 hr post-injury.

Within 24 hr post-injury, with a moderately severe lesion into the deeper connective tissue, the newly formed cellular sheet covers the wound and, further, fills the wound to the depth of the longitudinal muscle layer and organizes into new connective tissue (Fig. 3). By 48 hr post-injury, much debris is incorporated into the plug of new connective tissue, and the outermost layers of the plug slough (Fig. 4). Because the plug remains undiminished over time, it appears that it is replenished from below, and that considerable debris is surrounded and carried to the exterior by newly formed sacrificial connective tissue cells.

This sloughing process continues until all debris is eliminated, and at about 96 hr post-injury reepithelization begins by overgrowth from the periphery of the wound. Uninjured epithelial cells adjacent to the wound flatten somewhat, without becoming squamate, migrate over the connective tissue plug, and assume a generally cuboidal shape. The process is virtually complete after about 24 hr, or 120 hr post-injury. No mitotic figures were noted in epithelial cells. The plug itself, meanwhile, undergoes no change, remaining an undifferentiated mass of parenchymatous connective tissue.

Specimens sampled over 6 additional days exhibited no further repair. No tissue, therefore, is regenerated, and the wound cavity is

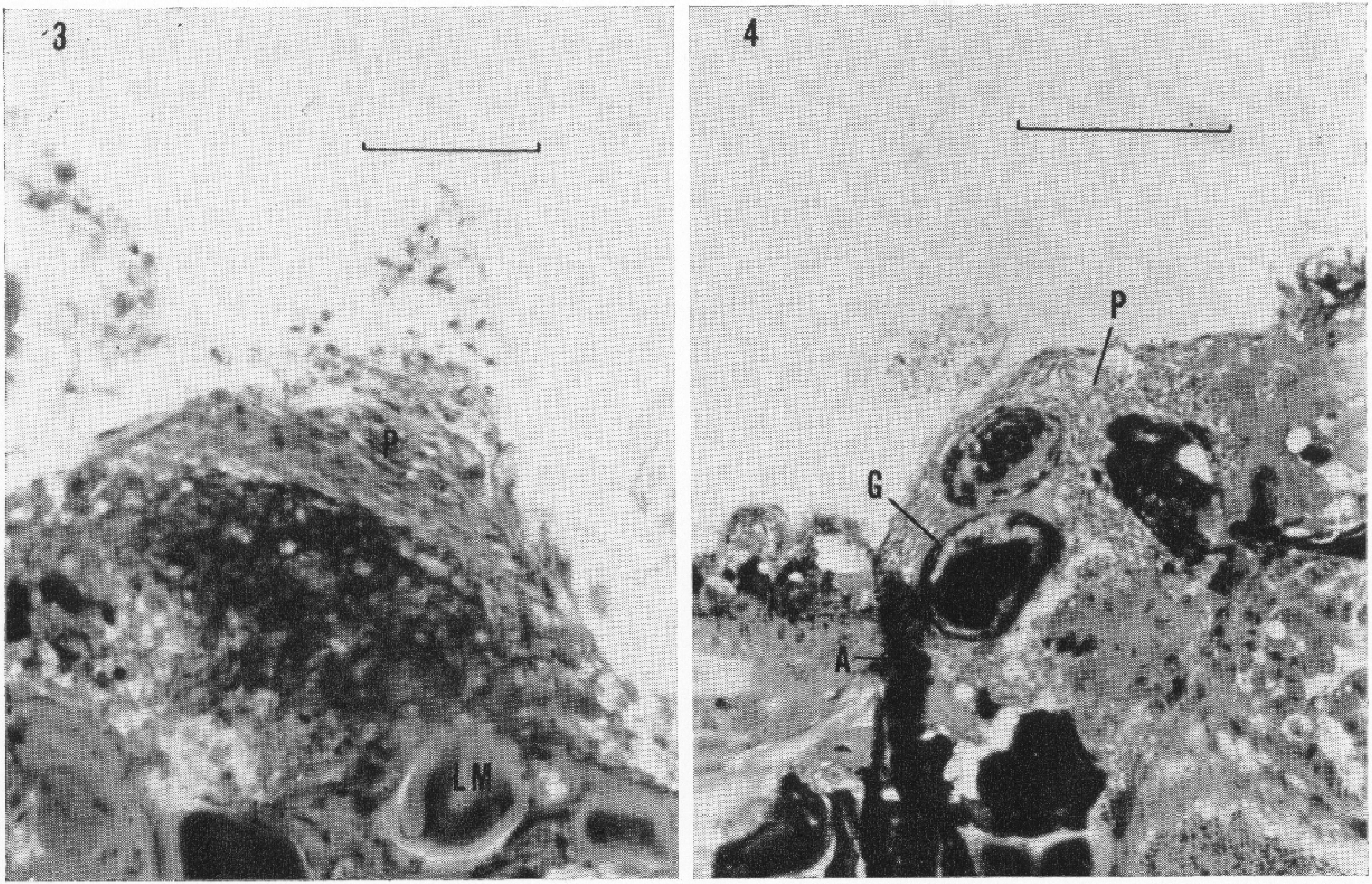


FIG. 3. 24 hr post-injury. A new connective tissue plug (P) fills the wound to longitudinal muscle (LM) depth. Note that the outer layers of the plug are sloughing. Line equals 0.050 mm (50 μ).

FIG. 4. 48 hr post-injury. Necrotic cocoon gland cells (G) and other debris are incorporated into the connective tissue plug (P). Note the apical sloughing layers of the plug. A–artifact. Line equals 0.150 mm (150 μ).

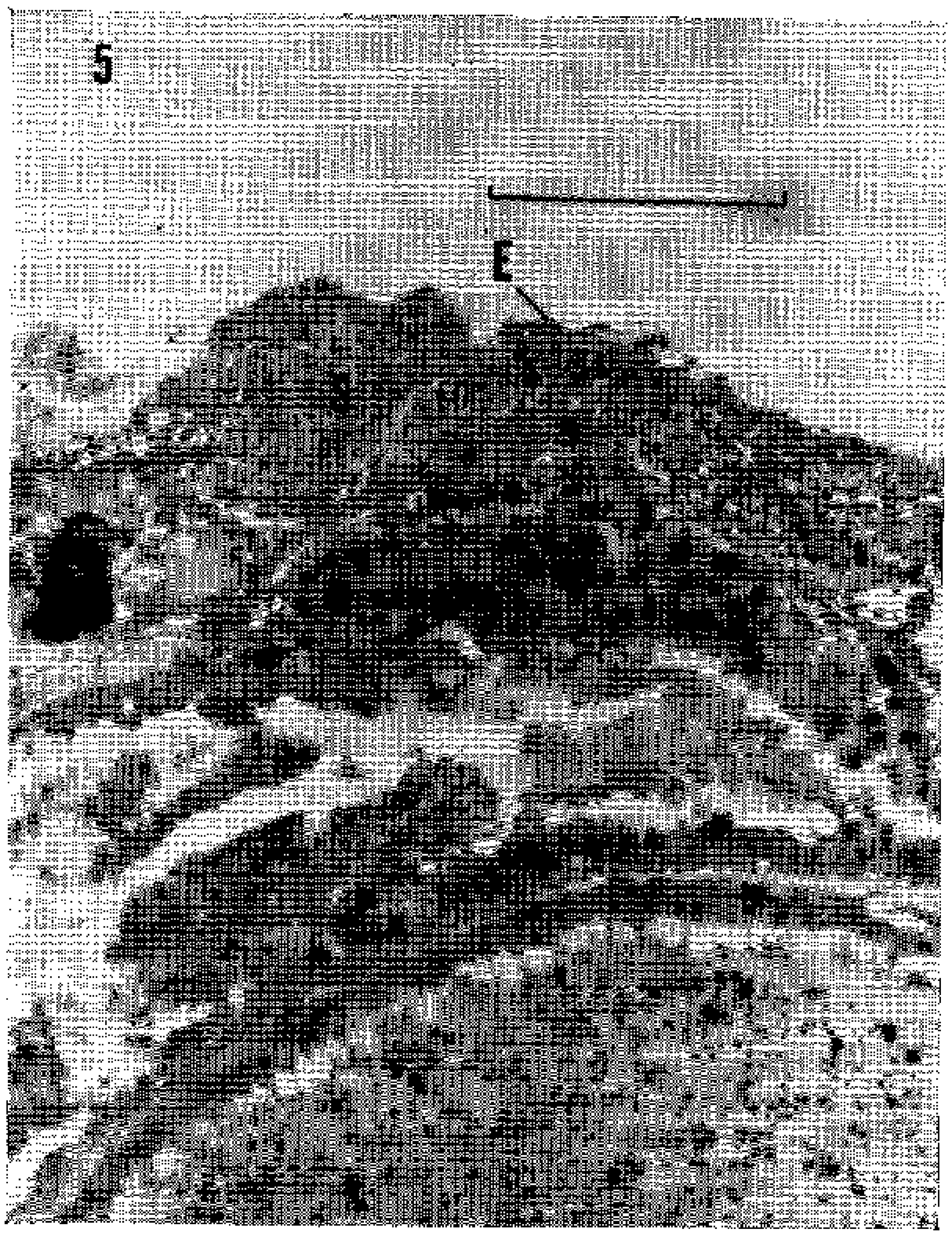


FIG. 5. 240 hr post-injury. A scar (S) of new connective tissue has formed and has been covered by epithelium (E). Note that the scar replaces the entire body wall. Line equals 0.150 mm (150 μ).

simply filled with connective tissue and covered with epithelium to form a scar (Fig. 5). Grossly the scar may appear lighter in color than the surrounding tissue because the fat, or pigment, cells are not replaced.

The few leeches that died during the experiments were processed, but examination of those not too decomposed for study proved enigmatic. The wounds inflicted were frequently less severe than those successfully healed by surviving leeches, were in the same area, and apparently no additional tissues were damaged. Stomachs of dead leeches almost always contained fish blood, indicating that death, or lack of response, was unrelated to nutrition.

DISCUSSION

Comparative invertebrate pathology has implied evolutionary development of cellular repair and defense processes, indicating that such studies may have considerable application to problems of human and veterinary medicine. Accordingly, *P. salmositica*'s response to injury may significantly be compared to that of the related polychaetous and oligochaetous annelids.

As expected, the initial response to injury is the same in the segmented worms and in *P. salmositica*, certainly because of the great similarity in body musculature. As soon as contraction of the area and sealing of the wound are completed, however, the similarity virtually ends. Whereas phagocytosis is a dominant defense mechanism in the worms, it is insignificant in *P. salmositica*, and, in fact, a major coelomic space apparently must be violated to elicit even this weak response. In the annelids, formation of the undifferentiated plug is largely independent of the debris-clearing mechanism, while in at least this species of leech, debris removal depends largely on plug formation. Indeed, even the origin of the plug cells is different; and, additionally, the worm plug may be referred to as a "blastema," because it is capable of differentiation into organized tissue, but the term must be withheld from *P. salmositica*, as no such activity is exhibited.

In summary, there is little similarity in the reaction to injury of the body wall of polychaete and oligochaete worms on the one hand, and of *P. salmositica* on the other. The one prominent exception to this is the process of cellular dedifferentiation, migration, and redifferentiation, demonstrated in *P. salmositica* by immigrating cells, which are most likely dedifferentiated connective tissue cells. Similar, but more generalized processes are described in oligochaetes by Krecker (1923) and Cameron (1932) and in polychaetes by Clark et al. (1962) and Herlant-Meewis and Van Damme (1962). Overall, *P. salmositica*'s ability to repair body wall wounds is decidedly inferior to, or degenerate from, that of polychaetous and oligochaetous annelids.

Acknowledgments

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